REMARKS/ARGUMENTS

Claims 1-17 were pending. By virtue of the present amendment, claims 9-17 have been canceled, and therefore, claims 1-8 are now pending. Applicant respectfully submits that the present application overcomes all prior rejections and has been placed in condition for allowance for the reasons set forth in detail below.

Priority

The Examiner indicates that priority should be referred to in the first sentence of the specification or in an application data sheet. At the time this case was filed, Applicant claimed priority in the declaration. Nonetheless, Applicant now amends this application to include reference to priority of the provisional application, and this removes any question with regard to the claim of priority.

Restriction Requirement

By virtue of Applicant electing to prosecute claims 1-8 in this application, Applicant has canceled claims 9-17 directed to the non-elected subject matter. Applicant reserves the right to file continuing applications directed to such matter.

Claim Rejections – 35 USC § 112, second paragraph

Claims 1-8 have been rejected as allegedly being indefinite for recitation of the term "a tightly regulated promoter." Applicant respectfully traverses the rejection.

Contrary to the Examiner's assertion that the specification does not define or teach specific features of these promoters, page 12, lines 10-30 of the specification, recites:

> Under specific conditions, these [tightly regulated] promoters operate to down regulate the production of the recombinant PAL mRNA, and consequently mitigate any detrimental effects on the host cell due to the production of the recombinant lipidated PAL (37). This tight regulation can then be removed under specific conditions to allow for maximized recombinant lipidated PAL expression in the host cell.
>
> These tightly regulated promoters (which may be together with other control elements) include, but are not limited to the arabinose inducible promoter (38), the T7 promoter which may be modified to be under control by nutL/N

antitermination function (39,40) or by Mu C (41), the P_L promoter in combination with antiterminator (42), the SP6 RNA polymerase and SP6 promoters (43), the colicin promoter (44), the tetA promoter/operator (45), the rhamnose and phosphate promoters (46), the LacR/O, tetR/O and AraCI1-12 regulatory elements (47), and invertible promoters (48).

By contrast, at page 3, lines 29-35, Applicant points out that previous attempts to express lipidated rP6 relied on promoters which were not under tight transcriptional regulation, such as trc, taq, lac (which was used in Anilionis et al; see next section) and P_L-C1857. These leaky promoters contributed to low levels of expression of the lipidated protein.

Therefore, since Applicant has defined and exemplified tightly regulated versus leaky promoters in the specification, Applicant respectfully requests that this rejection be withdrawn.

Claim Rejections – 35 USC § 102

Claims 1, 2 and 8 have been rejected under 35 USC 102(b) as allegedly being anticipated by Anilionis et al. (WO 90/02557) in light of Nelson et al. (*Infection and Immunity*, 56(1):128-134, 1988).[1]

Anticipation requires identity of invention. That is, each and every element of the claimed invention must be disclosed in a single cited reference, either expressly or inherently. Claim 1 is directed to a plasmid constructed to contain a tightly regulated promoter operatively linked to a gene that encodes a recombinant lipidated PAL. It is this plasmid construct that allows for the overexpression of lipidated PALs. The Anilionis plasmid does not contain a tightly regulated promoter, and therefore, does not anticipate the claimed invention.

While the protein in question in the Anilionis reference is a lipoprotein, the promoter utilized is the well-known and extensively studied *lac* promoter. This promoter is well known to those skilled in the art as a highly leaky promoter (i.e., it induces some level of protein expression in the absence of any induction). The best regulation of the *lac* promoter is accomplished by adding a mutated P-*lac* regulatory gene (LacIq) to the

[1] An anticipation rejection must be based on a single reference, not a combination of references. Applicant therefore addresses only Anilionis in this rejection. Besides, Applicant himself states in the specification at page 3, line 1, that P6 is also known as PBOMP-1; therefore, Nelson is of no moment.

plasmid backbone in an attempt to further control the *lac* promoter. Even this is insufficient to completely abolish some basal level expression of *lac* promoted genes. The cover page of the Anilionis reference contains a plasmid diagram that clearly shows that the fusion protein gene is under control of the *lac* promoter. No mention is made in the claims or the specification of the necessity of a tightly regulated promoter for optimal protein expression.

Applicant respectfully submits that Anilionis does not anticipate the claimed invention and requests that the rejection be withdrawn.

Claim Rejections – 35 USC § 103

Claims 3-5 have been rejected under 35 USC 103(a) as allegedly being obvious over Anilionis in light of Nelson, and in view of Guzman et al. (*Journal of Bacteriology*, 177(14):4121-4130, 1995).

Claims 3 and 6 have been rejected under 35 USC 103(a) as allegedly being obvious over Anilionis in light of Nelson, and in view of Mertens et al. (*Gene*, 164:9-15, 1995).

Claims 3, 6 and 7 have been rejected under 35 USC 103(a) as allegedly being obvious over Anilionis in light of Nelson, and in view of Mertens and Novagen Inc.

None of the cited combinations of references renders the claims obvious for the following reasons:

Anilionis (WO 90/02557) teaches peptides and proteins related to an outer membrane protein (OMP) of about 16,000 daltons molecular weight of *Haemophilus influenzae* identified as PBOMP-1 and PBOMP-2. Anilionis also teaches fusion proteins of PBOMP-1 and PBOMP-2. In fact, the lipoprotein expressed in Anilionis is a fusion protein of the P4 and P6 OMPs of *H. influenzae*. The promoters disclosed in Anilionis include, for example, lac, trp, recA, ribosomal RNA, the P_R and P_L promoters of coliphage lambda and others including, but not limited to, lacUV5, ompF, bla, lpp and the hybrid trp-lacUV5 (tac). None of these promoters are tightly regulated promoters; instead, they are well known in the art as leaky promoters.

Nelson et al. cloned the gene encoding P6, a 16,600-dalton protein present in the outer membranes of both typeable and nontypeable strains of *H. influenzae*, expressed the P6 polypeptide in *E. coli*, and sequenced the gene encoding P6.

Guzman et al. constructed vectors (pBAD vectors) containing the tightly regulated P_{BAD} promoter of the arabinose operon and its regulatory gene, *araC*.

Mertens et al. cloned the T7*g10* gene and analyzed the expression characteristics using a clockwise oriented λ P_L-containing expression vector. Expression-promoting elements from the latter gene were subsequently used for construction of a dual-promoter expression plasmid, containing both λ P_L and P_{T7} promoters, for heterologous gene expression in *E. coli*.

Novagen Inc. supplies the commercially available expression vector pET27b which has the T7 promoter.

As to the rejection of claims 3-5, the Examiner asserts that it would have been obvious to the skilled artisan to subclone PBOMP-1 (i.e., P6) into any of the arabinose inducible vectors of Guzman because Anilionis teaches that it is desirable to use strong promoters to obtain a high level of transcription. As to the rejection of claims 3 and 6, the Examiner asserts that it would have been obvious to subclone PBOMP-1 into any of the PT7 containing vectors of Mertens because Anilionis teaches that it is desirable to use strong promoters to obtain a high level of transcription, and Mertens teaches that the PT7 containing vectors have the *potential* to improve the expression level of other heterologous genes. As to the rejection of claims 3, 6 and 7, the Examiner asserts it would have been obvious to subclone PBOMP-1 into the commercially available vector pET-27B of Novagen because Anilionis teaches that it is desirable to use strong promoters to obtain a high level of transcription.[2]

Applicant traverses these rejections because in each instance the Examiner's rejection lacks the necessary substantial evidence to support a rejection of Applicant's claims.

Most if not all inventions arise from a combination of old elements. *See In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453,1457 (Fed. Cir. 1998). Thus, every element of a claimed invention may often be found in the prior art. *See id.* However, identification in the prior art of each individual part claimed is insufficient to defeat patentability of the whole claimed invention. *See id.* Rather, to establish obviousness

[2] Note that the terms "tight" and "strong" with respect to promoters are not synonymous. "Tight" refers to the activation of the promoter, i.e., it is either on or off. "Strong", on the other hand, refers to the level of transcription. A "strong" promoter would therefore be used to obtain a high level of transcription.

based on a combination of elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant. *See In re Dance*, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998); *In re Gordon*, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Cir. 1984). It is impermissible to use the claims as a framework from which to pick and choose among individual references to recreate the claimed invention. *See In re Fine*, 5 USPQ2d 1586, 1600 (Fed. Cir. 1988).

The motivation, suggestion or teaching may be found in explicit or implicit teachings within the references themselves, from the ordinary knowledge of those skilled in the art, or from the nature of the problem to be solved. *See WMS Gaming, Inc. v. International Game Tech.*, 51 USPQ2d 1385, 1397 (Fed. Cir. 1999). However, there still must be **evidence** that "a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed." *In re Rouffet*, 47 USPQ2d at 1456; *see also In re Kotzab*, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000) ("[a] rejection cannot be predicated on the mere identification . . . of individual components of claimed limitations. Rather, particular findings must be made as to the reason the skilled artisan, *with no knowledge of the claimed invention*, would have selected these components for combination in the manner claimed.") (Emphasis added.)

Here, no such evidence was presented. Rather than pointing to specific information in Nelson, Guzman, Mertens or Novagen that suggests their combination with Anilionis to yield the claimed invention, the Examiner merely discusses how the cited references can be combined to read on the claimed invention. This reference-by-reference, limitation-by-limitation analysis wholly fails to demonstrate how the cited references teach or suggest the combination claimed in the present invention. *In re Dembiczak*, 50 USPQ2d 1614, 1618 (Fed. Cir. 1999).

Moreover, consistent with the rule that all evidence of nonobviousness must be considered when assessing patentability, the Examiner must consider that previous attempts by those skilled in the art to overexpress lipidated P6 failed. As disclosed in the present specification:

> Several laboratories were unable to express lipidated rP6 (also known as PBOMP-1) in large quantities in *E. coli* (28,29). As a result, initial recombinant

constructs expressing P6 in *E. coli* could express only a nonlipidated version of the protein. (Page 3, lines 18-22)

Previous [unsuccessful] attempts to express lipidated rP6 relied on promoters which were not under tight transcriptional regulation, such as trc, taq, lac and P_L-C1857. (Page 3, lines 29-31)

Previous unsuccessful efforts to express lipidated P6 protein in meaningful quantities for commercial use all relied on changing the promoter sequence and/or making changes in the signal sequence recognized by signal peptidase II. (Page 8, lines 3-7)

Further evidence that leaky promoters will not result in an overexpression of lipidated P6 can be found in the Anilionis reference itself. Example 8 of Anilionis discloses that "[w]hen PBOMP-1 was expressed from *lac* or P_L promoters in *E. coli* JM103 or HB101 strain, only low levels of PBOMP-1 were expressed." (Emphasis added.)

So even though the individual elements of the claimed invention were known in the art, no one until Applicant figured out what combination would work. In contrast to previous efforts that failed, the plasmid construct of the present invention was, and still is, the first construct that allows for significant expression of the lipidated P6 protein.

Based on the foregoing, Applicant respectfully submits that the Examiner has failed to establish a *prima facie* case of obviousness. Consequently, the rejections are improper and should be withdrawn.

Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

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